SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of L-valine produced by *Escherichia coli* NITE SD 00066 for all animal species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The additive L-valine is produced by a strain of *Escherichia coli* (NITE SD 00066) obtained by chemical mutagenesis. The additive L-valine is safe for all animal target species/categories when used in appropriate amounts to supplement feed to compensate for a valine deficiency in feedingstuffs. The composition of tissues and products of animal origin will not be changed by the use of L-valine in animal nutrition. Considering the high purity of the product under assessment, no risks are expected for the consumer from its use as a feed additive. In the absence of any data, it would be prudent to consider L-valine produced by *E. coli* NITE SD 00066 to be an irritant to skin, eyes and mucous membranes, a potential dermal sensitiser and potentially harmful by inhalation. The amino acid L-valine is a natural component of plants and animals. The use of the product L-valine under assessment in animal nutrition does not represent a risk to the environment. The product L-valine is considered to be an efficacious source of the amino acid L-valine for all non-ruminant species. Supplementary free amino acid valine is degraded by ruminal microorganisms if it is not given in a protected form.

KEY WORDS

nutritional additives, amino acids and their salts and analogues, L-valine, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2012-00694, adopted on 9 December 2014.

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on L-valine (98 %) produced by fermentation using a traditionally modified strain of *Escherichia coli* (NITE SD 00066) for all animal species.

L-valine is an essential amino acid that may become limiting under specific feeding conditions. Its supplementation has become even more important since protein-reduced diets were introduced in animal husbandry for economic and environmental reasons. In European vegetable feed formulas, L-valine seems to be the fifth most limiting amino acid after L-tryptophan in pigs for fattening and fourth after L-threonine in chickens for fattening.

The additive L-valine is safe for all animal target species/categories when used in appropriate amounts to supplement feed to compensate for a valine deficiency in feedingstuffs.

The composition of tissues and products of animal origin will not be changed by the use of L-valine in animal nutrition. Considering the high purity of the product under assessment, no risks are expected for the consumer from its use as a feed additive.

In the absence of any data, it would be prudent to consider L-valine produced by *E. coli* NITE SD 00066 to be an irritant to skin, eyes and mucous membranes, a potential dermal sensitiser and potentially harmful by inhalation.

The amino acid L-valine is a natural component of plants and animals. The use of the product L-valine under assessment in animal nutrition does not represent a risk to the environment.

The product L-valine is considered to be an efficacious source of the amino acid L-valine for all non-ruminant species. Supplementary free amino acid valine is degraded by ruminal microorganisms if it is not given in a protected form.

The FEEDAP Panel has made a recommendation regarding the product description.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company Kyowa Hakko Europe GmbH[5] for authorisation of the product L-valine (98 %) produced by *Escherichia coli* NITE SD 00066 when used as a feed additive for all animal species (category: nutritional additives; functional group: amino acids, their salts and analogues) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[6] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 12 November 2012.

The additive under application L-valine is produced using *Escherichia coli* NITE SD 00066. It has not been previously authorised as a feed additive in the European Union.

L-Valine is one of the substances listed in Annex III of Commission directive 2006/141/EC and therefore authorised for the manufacture of infant formulae and follow-on formulae in order to satisfy the requirements on amino acids and other nitrogen compounds. In a "pharmaceutical" grade form it is used for parenteral nutrition.[7] It may also be added for specific nutritional purposes in foods for particular nutritional uses according to Commission Directive 2001/15/EC.[8] L-Valine and DL-valine are also authorised as sensory additives, belonging to the functional group flavouring compounds (FLAVIS No 17.028 and 17.023, respectively).[9] L-Valine has a dedicated monograph in the European Pharmacopoeia (EurPh).[10]

The product L-valine produced by *E. coli* FERM ABP-10640, currently authorised as nutritional additive until 3rd of June 2019,[11] was assessed by the FEEDAP Panel of EFSA in 2008 (EFSA, 2008a,b). The EFSA FEEDAP Panel issued two scientific opinions on the safety and efficacy of L-valine for all animal species, one produced by *Corynebacterium glutamicum* KCCM 80058 and another by *C. glutamicum* DSM 25202 (EFSA FEEDAP Panel, 2013, 2014). The safety of L-valine when used as food flavouring was assessed by Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) and by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) (EFSA, 2008c).

[4] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[5] Kyowa Hakko Europe GmbH, Am Wehrhahn 50, D-40211, Düsseldorf (Germany).
[6] EFSA Dossier reference: FAD-2012-0023.
[7] Commission Regulation (EC) 403/2009 of 14 May 2009 concerning the authorisation of a preparation of L-valine as feed additive. OJ L 120, 15.05.2009, p. 2.
[8] Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 52, 22.2.2001, p 1–7.
[9] Commission list of the authorised additives in feedingstuffs published in application of Article 9t(b) of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01) OJ C/50, 25.2.2004, p 1–144.
[10] EurPh monograph 01/2008: 0796, correction 6.0.
[11] Commission Regulation (EC) 403/2009 of 14 May 2009 concerning the authorisation of a preparation of L-valine as feed additive. OJ L 120, 15.05.2009, p. 2.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of the product L-valine (L-valine 98 %) produced by *Escherichia coli* NITE SD 00066, when used under the conditions described in Table 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	L-Valine			
Registration number/EC No/No (if appropriate)				
Category(ies) of additive	Nutritional			
Functional group(s) of additive	amino acids, their salts and analogues.			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
L-Valine produced by *Escherichia coli* (NITE SD 00066)	$C_5H_{11}NO_2$	98%	determination of amino acids as set out in Commission Regulation (EC) No 152/2009	
Trade name (if appropriate)	N/A			
Name of the holder of authorisation (if appropriate)	N/A			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All species	-	-	-	-
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	-			
Specific conditions or restrictions for handling (if appropriate)	-			
Post-market monitoring (if appropriate)	-			
Specific conditions for use in complementary feedingstuffs (if appropriate)	-			
Maximum Residue Limit (MRL) (if appropriate)				
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues	
-	-	-	-	

ASSESSMENT

The application contains data from a single source of L-valine obtained by fermentation using a strain of *Escherichia coli* (NITE SD 00066) obtained by chemical mutagenesis. The product under application is intended to be used in feed for all animal species.

This opinion is based in part on data provided by a single company involved in the production/distribution of L-valine. It should be recognised that these data cover only a fraction of the existing additives containing L-valine. The FEEDAP Panel has sought to use the data provided, together with data from other sources, to deliver an opinion.

1. Introduction

L-Valine is an essential proteogenic amino acid belonging, along with leucine and isoleucine, to the group of branched-chain amino acids (BCAAs). The objective of feed supplementation with essential amino acids is to complete the amino acid profile of the diet in order to more closely meet the requirements of the animals for individual amino acids. The supplementation of feedingstuffs with amino acids is a conventional measure to improve protein quality and utilisation. This supplementation has become even more important since protein-reduced diets were introduced in animal husbandry for economic and environmental reasons. It is well recognised that L-valine as an essential amino acid may become limiting under specific feeding conditions.

2. Characterisation

The FEEDAP Panel notes that all data necessary to characterise the product are based on pilot batches, as large-scale industrial production has not yet started. The data can only be regarded as representative of the commercial product if large-scale manufacturing would result in a product identical to the pilot product. Consequently, the following assessment would only apply if these conditions are met.

2.1. Characterisation of the active substance/additive

L-Valine (International Union of Pure and Applied Chemistry (IUPAC) name (2S)-2-amino-3-methylbutanoic acid; synonyms α-aminoisovaleric acid, 2-amino-3-methylbutyric acid), a compound identified by the Chemical Abstracts Service (CAS) No 72-18-4 and the European Inventory of Existing Commercial chemical Substances (EINECS) No 200-773-6, is an essential amino acid.[12] It has a molecular weight of 117.15 g/mol; the molecular formula is $C_5H_{11}NO_2$ and the structural formula is given in Figure 1.

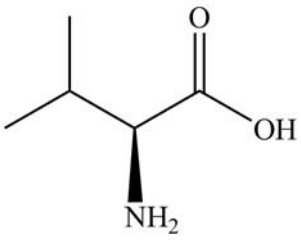

Figure 1: Molecular structure of valine

By specification, the product contains ≥ 98 % L-valine, 'as is', and loss on drying is ≤ 1 %. Analysis of five product batches by the European official method shows an average valine content of 99.6 % (range 98.5 to 101.2 %) on a dry matter basis. The mean loss on drying was within a range 0.09 to 0.18 %, and ash (residue on ignition) was in the range 0.09 to 0.15 %.[13] Further analysed constituents were other amino acids (on an 'as is' basis) including isoleucine (up to 0.067 %), leucine (up to

[12] Technical dossier/Section II.2.1.3.
[13] Supplementary information March 2014/Annexes A and B.

0.049 %), phenylalanine (up to 0.064 %) and tyrosine (up to 0.082 %). The sum of cysteine + cystine (found above a limit of quantification (LOQ) of 0.006 % in only four batches) was up to 0.014 %, whereas alanine (found above a LOQ of 0.015 % only in one batch) was 0.021 %.[14] Thus the calculated average amount of unidentified material is 0.06 % on a dry matter basis, except in one batch (1.13 %). No other substances were analysed in the product for the identification of unknown material. The analysed specific optical rotation at 20 °C ranges between +27.9 and +28.0 °.[15] These values comply with the reference values for L-valine (European Pharmacopoeia:[16] +26.5 to +29.0 °; Kleemann et al. (1985): +26.7 °; Weast (1975): +28.8 °).

2.2. Impurities

Analysis of the same five batches described above showed concentrations of heavy metals (expressed as lead) and arsenic below 10 and 1 mg/kg, respectively.[17] In three additional batches, very low concentrations of Cd (≤ 0.01), Hg (≤ 0.01), Pb (≤ 0.05) and As (≤ 0.1 mg/kg) were found. Residual minerals (Na ≤ 567, K ≤ 160, Mg ≤ 23 mg/kg) and trace elements (Fe ≤ 2.7, Cu ≤ 0.06, Mn ≤ 0.16, Zn ≤ 0.28, Se ≤ 0.05 mg/kg) were also analysed in these batches. Concentrations of ammonium, chloride and sulphate were < 0.012, ≤ 0.017 and ≤ 0.08 %, respectively.[18]

No viable bacteria cells of the production strain were detected in three batches of the final product by microbiological analysis.[19] *Salmonella* was absent (tested in 25 g of the product). Three batches of the final product were tested for the presence of coliform bacteria, filamentous fungi and yeasts, and all results were negative. Enterobacteriaceae and aerobic plate counts were 0.3 and < 300 colony forming units (CFU)/g, respectively. Aflatoxin B1 and ochratoxin A were below the LOQ (5 µg/kg).[20]

2.3. Physical state of the product

The additive is a grey-white to grey yellow-brown crystalline powder or granules. The reported effective particle density is 1.27 g/cm^3.[21]

Particle size distribution analysed in one batch of the additive by laser diffraction analysis showed 9.1 and 0.91 % (v/v) particles with a diameter below 100 and 50 µm, respectively, and no particles were smaller than 10 µm. In two other batches no particles below 100 µm were found.[22] No data on dusting potential were provided.

2.4. Characterisation of the production organism[23]

L-Valine is produced by fermentation with a chemically mutated *E. coli* strain, which is deposited at the National Institute of Technology and Evaluation (NITE) Biological Resource Center, Japan, with accession number NITE SD 00066.[24]

The production strain was confirmed to be *E. coli* by sequence comparison of 16S rRNA genes.[25] *E. coli* NITE SD 00066 is a derivative of *E. coli* W type, which is generally regarded as safe and its genome sequence is available (Archer et al., 2011). The production strain does not raise concerns regarding its capacity to produce substances with antimicrobial activity or toxins. The technical

[14] Supplementary information March 2014/Annex A.
[15] Supplementary information June 2013/Annex B.
[16] EurPh monograph 01/2008: 0796, correction 6.0.
[17] Supplementary information March 2014/Annex B.
[18] Supplementary information June 2013/Annex C.
[19] Supplementary information June 2013/Annex E.
[20] Supplementary information June 2013/Annexes E and F.
[21] Technical dossier/Section II.1.4/Table II.2.
[22] Technical dossier/Section II/Annex II.4 and supplementary information June 2013/Annex D.
[23] This section has been amended following the confidentiality claims made by the applicant.
[24] Supplementary information June 2013/Annex I.
[25] Supplementary information October 2014/Annexes A and B.

dossier contains information demonstrating the absence of potential of the production strain to produce toxins and virulence factors. [26]

Regarding the antibiotic sensitivity of the production strain, no data on the minimum inhibitory concentration to the antibiotics listed in the technical guidance on the assessment of bacterial antimicrobial susceptibility (EFSA FEEDAP Panel, 2012) were provided. Nevertheless, the applicant demonstrated the absence of DNA of the production strain in three batches of the final product.[27]

Based on the absence of antimicrobial activity, toxins and virulence factors, the production strain *E. coli* NITE SD 00066 is safe for the production of L-valine.

2.5. Manufacturing process[28]

L-Valine is produced by fermentation of the production strain. After the fermentation step, the fermentation broth is inactivated and L-valine is purified and crystallised.

Material safety data sheets of the materials used in the manufacture and purification process and of the final product are provided.

The applicant declared that no antibiotic substances are used during the manufacturing process.[29]

2.6. Stability and homogeneity

2.6.1. Shelf life of the additive

When stored in closed containers under ambient conditions (temperature not specified), three product batches showed no losses of valine after 42 months.[30] In accelerated conditions (temperature 40 °C), the same batches stored in closed containers showed full recovery of valine after six months.[31] However, these data were not obtained by the EU official analytical method for valine.

2.6.2. Stability in premixtures

The stability of one batch of valine (15 % inclusion) was studied in three different commercial vitamin (without choline chloride)/trace elements premixtures for piglets, pigs for fattening and chickens for fattening. The premixtures were stored in polyethylene bottles (temperature not specified). No losses of valine were found after seven months.[32]

2.6.3. Stability in compound feed

The stability of L-valine (one batch) was tested in both mash and pelleted complete feeds for pigs for fattening (based on barley, wheat and canola) and chickens for fattening (based on maize, wheat and soybean meal) with an intended supplementation rate of 0.2 %. After three months' storage in polyethylene bottles at ambient temperature, the losses in mash feed for pigs and chickens for fattening were 19 and 15 %, respectively. In pelleted feed for pigs for fattening and chickens for fattening the valine content was reduced after the same period by 2 and 7 %, respectively. The pelleting procedure (steam pressure 2 bar, pellet size 3 mm, temperature 70 ± 5 °C) with the same feeds as above did not affect the valine content in complete feed for pigs for fattening, whereas a loss of 8.5 % was found in complete feed for chickens for fattening (one batch of additive tested). [33]

[26] Supplementary information June 2013/Annex G.
[27] Supplementary information October 2014/Annexes C, D and E.
[28] This section has been amended following the confidentiality claims made by the applicant.
[29] Supplementary information October 2014/Annex F.
[30] Supplementary information June 2013/Annex J.
[31] Supplementary information June 2013/Annex J.
[32] Technical dossier/Section II/Annex II.10.
[33] Technical dossier/Section II/Annex II.10.

The FEEDAP Panel notes that stability data in feeds were calculated from total valine analysed with a correction for protein-bound valine.[34] Therefore these data may not fully represent the stability of added free L-valine in feed.

2.6.4. Homogeneity

The potential of the additive to distribute homogeneously in feed was tested (free valine calculated from total valine analysed and corrected for protein-bound valine) immediately after pelleting of mash complete feed for chickens for fattening (same feed as above with 0.2 % inclusion of L-valine). The analysis of 10 subsamples of pellets showed a coefficient of variation of 6.1 %. However, data on the concentration of total valine do not allow the full assessment of the ability of the additive to homogeneously distribute in compound feedingstuffs.[35]

2.7. Incompatibilities

No physico-chemical incompatibilities in feed are expected when used with other additives, medicinal products or other feed materials.

2.8. Conditions of use

L-Valine is intended to be used in feed for all animal species and categories with no minimum or maximum content specified. No withdrawal period is proposed. The need to supplement L-valine generally depends on the requirement of the animal species/categories and the background content of this essential amino acid in feedingstuffs. It is proposed that L-valine be supplemented to feedingstuffs via premixtures or directly into complete feed or complementary feed.

2.9. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of the L-valine in animal feed. The Executive Summary of the EURL report can be found in the Appendix.[36]

3. Safety

As a general principle, conventional toxicology studies are considered to be inappropriate for amino acids. Dietary intakes of amino acids that lead to amounts significantly below or above that which is optimum for health and performance will inevitably cause a physiological imbalance and consequently adverse effects.

3.1. Safety for the target species

Tolerance studies are not normally required for highly purified amino acids. Such tolerance studies with a certain indispensable amino acid will inevitably result in amino acid imbalances, with depression of feed intake and hence impaired performance. This is also the case for the product under application, which contains ≥ 98.5 % valine and an average content of less than 1 % unidentified material. It is expected that large-scale production would be optimised to achieve less than 1 % unidentified material in every batch. Therefore, the FEEDAP Panel considers that safety concerns for target species are highly unlikely to arise from this additive.

The requirements of target animal species for valine and the safety of using this essential amino acid in animal nutrition were recently summarised in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2013). The previous opinion did not include the requirements of fish for valine; these range from 0.6 to 1.7 % in different fish species (for a detailed review see NRC, 2011).

[34] Technical dossier/Section II/Annex II.10.
[35] Technical dossier/Section II/Annex II.10.
[36] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2012-0023-L-Valine.doc.pdf

3.1.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that the additive L-valine produced by *E. coli* NITE SD 00066 is safe for all animal species/categories. However, excess doses would create amino acid imbalances with negative consequences for animal performance. Correct dosing in formulating diets requires knowledge of the amino acid content in feed materials and the requirement of animals.

3.2. Safety for the consumer

Absorption, distribution, metabolism and excretion of the amino acid L-valine have been described in a previous EFSA scientific opinion (EFSA FEEDAP Panel, 2013).

As a general principle, conventional toxicology studies are considered to be inappropriate for amino acids. Dietary intakes of amino acids that lead to amounts significantly below or above that which is optimum for health and performance will inevitably cause a physiological imbalance and consequently adverse effects.

The product under assessment is produced by fermentation. Concerns for the consumer would derive not from the amino acid itself, which will be incorporated into protein (the potential excess will be metabolised and excreted as urea/uric acid and carbon dioxide) but from possible residues from the fermentation. In this case, the additive is highly purified (> 98.5 % L-valine and < 1 % unidentified material on dry matter basis) and, therefore, following the provisions of the guidance on nutritional additives, it is considered to be of no safety concern for consumers.

3.2.1. Conclusions on safety for the consumer

As the composition of tissues and products of animal origin will not be changed by the use of L-valine in animal nutrition, and considering the high purity of the product under assessment, the use of L-valine produced by *E. coli* NITE SD 00066 in animal nutrition is of no safety concern for consumers.

3.3. Safety for the user

3.3.1. Effects on the respiratory system

The particle size distribution (laser diffraction) of three batches of the additive showed that the percentages of the additive having a diameter of less than 50 μm were 0.91, 0.0 and 0.0 %, respectively, with no particles of less than 10 μm diameter. No data were provided regarding the dusting potential or respiratory toxicity. Although the data on particle size would exclude the potential of fine particles to reach the respiratory surface of the lungs when inhaled, it does not exclude the possibility that other presentations with a different particle size distribution and dusting potential might be produced.

As the additive is produced by a Gram-negative microorganism, the presence of endotoxins cannot be excluded. Therefore, appropriate protective measures to minimise exposure of the respiratory tract to these substances are indicated.

3.3.2. Effects on the skin and eyes

As no studies were provided to examine the product for skin or eye irritancy or for dermal sensitisation, the product should be considered to be an irritant to both skin and eyes and a potential dermal sensitiser.

3.3.3. Conclusions on safety for the user

The particle size of the three batches analysed indicates that extensive respiratory exposure of the lungs is unlikely to occur. Nevertheless, as the possibility exists that other presentations with a different particle size distribution could be produced, respiratory exposure cannot be totally excluded. Considering that the presence of endotoxins cannot be excluded, protection measures to minimise

inhalation exposure are indicated. As no studies relevant to other aspects of the user safety assessment were provided, the FEEDAP Panel concludes that the product under application should be considered to be an irritant to both eyes and skin and a potential dermal sensitiser.

3.4. Safety for the environment

The amino acid L-valine is a physiological and natural component of animal and plant proteins. It is not excreted as such (but as urea/uric acid and carbon dioxide). The use of L-valine in animal nutrition would not lead to any localised increase in its concentration in the environment. Therefore the use of this product as a feed additive does not represent a risk to the environment.

4. Efficacy

Efficacy studies are not required for amino acids that occur naturally in plant and animal proteins. The nutritional role of the amino acid L-valine is well established in the scientific literature. The product L-valine is regarded as an efficacious source of the essential amino acid L-valine for non-ruminant nutrition.

In ruminants, the amino acid valine has been implicated as being present at lower than optimum levels in microbial protein leaving the rumen (O'Connor et al., 1993; Schwab et al., 2005). Thus, when requirements for more limiting essential amino acids, usually L-methionine, L-lysine and L-histidine, have been met, L-valine supplementation could be beneficial. Free L-valine is rapidly degraded by ruminal microbiota, with an estimated half-life in the rumen of 2.1 hours (Chalupa, 1976). Broderick and Balthrop (1979) found that 45 % of free L-valine added to ruminal digesta *in vitro* remained after 3 hours. Accordingly, only small amounts of dietary L-valine provided to ruminants would be expected to reach the abomasum intact and be absorbed. Therefore, measures such as encapsulation would ensure a more efficient delivery of L-valine beyond the rumen, and only limited nutritional benefit may be derived from dietary supplementation with the unprotected, free amino acid.

4.1. Conclusions on efficacy

The additive L-valine produced by *E. coli* NITE SD 00066 is regarded as an efficacious source of the essential amino acid L-valine for all non-ruminant species. Supplementary free amino acid valine is degraded by ruminal microorganisms if it is not given in a protected form.

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[37] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The additive L-valine is safe for all animal target species/categories when used in appropriate amounts to supplement feed to compensate for a valine deficiency in feedingstuffs.

The composition of tissues and products of animal origin will not be changed by the use of L-valine in animal nutrition. Considering the high purity of the product under assessment, no risks are expected for the consumer from its use as a feed additive.

In the absence of any data, it would be prudent to consider L-valine produced by *E. coli* NITE SD 00066 to be an irritant to skin, eyes and mucous membranes, a potential dermal sensitiser and potentially harmful by inhalation.

[37] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

The amino acid L-valine is a natural component of plants and animals. The use of the product L-valine under assessment in animal nutrition does not represent a risk to the environment.

The product L-valine is considered to be an efficacious source of the amino acid L-valine for all non-ruminant species. Supplementary free amino acid valine is degraded by ruminal microorganisms if it is not given in a protected form.

RECOMMENDATIONS

The description of the additive should contain the statement "produced by fermentation with *Escherichia coli* NITE SD 00066."

DOCUMENTATION PROVIDED TO EFSA

1. L-Valine—98 %. June 2012. Submitted by Kyowa Hakko Europe GmbH.
2. L-Valine—98 %. Supplementary information. June 2013. Submitted by Kyowa Hakko Europe GmbH.
3. L-Valine—98 %. Supplementary information. November 2013. Submitted by Kyowa Hakko Europe GmbH.
4. L-Valine—98 %. Supplementary information. March 2014. Submitted by Kyowa Hakko Europe GmbH.
5. L-Valine—98 %. Supplementary information. October 2014. Submitted by Kyowa Hakko Europe GmbH.
6. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for L-valine .
7. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for L-valine[38]

In the current application authorisation is sought under Article 4(1) for *L-valine* produced by *Escherichia coli* (NITE SD 00066), under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for *all animal species*. The *feed additive* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs*. The Applicant proposed no minimum or maximum *L-valine* concentrations in *feedingstuffs*, suggesting, however, typical levels of inclusion up to 2000 mg/kg complete feed.

For the determination of *L-valine* in *premixtures* and *feedingstuffs* the Applicant submitted the Community method - Commission Regulation (EC) No 152/2009 (further ring-trial validated - CEN EN ISO 13903:2005). The following performance characteristics were reported for the determination of *total valine*:

- a relative standard deviation for *repeatability* (RSDr) ranging from 1.7 to 3.8%; and
- a relative standard deviation for *reproducibility* (RSDR) ranging from 8.8 to 16%.

For the determination of L-valine in the *feed additive*, the Applicant submitted the European Pharmacopoeia general method for the determination of amino acids, based on High Performance Liquid Chromatography (HPLC). The EURL recommends instead the "*L-valine* monograph" of the Food Chemical Codex (FCC), where identification is based on infrared absorption in combination with the analysis of the optical rotation, while quantification is based on titration. Moreover, as already recommended in the report FAD-2007-0015, the EURL suggests the above mentioned Community method for the determination of *valine* in the *feed additive*.

Based on the performance characteristics presented, the EURL recommends for official control:

- the Food Chemical Codex for the determination of *L-valine* in the *feed additive* and
- the ring-trial validated Community method, based on ion exchange chromatography coupled with post-column derivatisation and photometric detection to determine valine in *feed additive*, *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

[38] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2012-0023-L-Valine.doc.pdf